$\delta^{13}C$ GC-IRMS CHARACTERISATION OF EXTRACTABLE AND COVALENTLY-BOUND ALIPHATIC HYDROCARBONS IN PETROLEUM SOURCE ROCKS TO REVEAL COMPOSITIONAL FRACTIONATION EFFECTS.

Gordon D. Love(1), Colin E. Snape(1), Anthony E. Fallick(2) and Christopher Taylor(2).
(1) University of Strathclyde, Dept. of Pure & Applied Chemistry, Thomas Graham Building, 295 Cathedral St., Glasgow G1 1XL, UK.
(2) Scottish Universities Research & Reactor Centre (SURRC), East Kilbride, Glasgow G75 OQU, UK.

Keywords : stable isotope geochemistry, kerogen formation, selective preservation .

INTRODUCTION

The structural elucidation of sedimentary fossil organic matter at a molecular level remains a challenging task, on account of its heterogeneous, largely insoluble nature. An elaborate sequential extraction/degradation scheme to differentiate between molecular alkanes (both easily extractable, and those physically-trapped or clathrated within the macromolecular structure) and alkyl moieties covalently bound within the kerogen network, was recently devised by the Strathclyde group. Briefly, the scheme incorporates exhaustive solvent extraction with dichloromethane (DCM) followed by pyridine to remove physically trapped material and then a mild batch hydrogenation step is performed to cleave weak heteroatomic bonds (principally ester, thioether and possibly ether linkages). The key and final stage in this procedure to release strongly-bound hydrocarbons is pyrolysis at high hydrogen pressures (up to 15 MPa, about 150 atmospheres) which is known as hydropyrolysis. Thermolysis in a high hydrogen pressure (reducing) environment eliminates the problem of low yields often associated with the use of sterically-bulky chemical reagents and limits the extent of retogressive, char-forming chemistry. Hydropyrolysis gives rise to overall carbon conversions approaching 100% for Type I and Type II kerogens (these have higher H/C ratios than coals, which are classified as Type III kerogens on the basis of elemental composition) with low hydrocarbon gas yields.

The attractiveness of the scheme lies in the fact that it recognises that the structure of sedimentary organic matter is three dimensional, comprising an organic macromolecular network (kerogen) in which material is trapped with differing degrees of mobility. The increase in severity of the reaction conditions as the sequence proceeds, produces a series of soluble products whose constitution is determined by the strength of association of structural units. The differences in the yield and distribution of selected classes of aliphatic hydrocarbons (such as biomarkers) produced from sequential degradation of a series of vitrinite concentrates [1] and petroleum source rocks [2] are reported elsewhere.

The carbon isotopic signature of the bulk organic matter and individual molecules can be helpful in determining the types of organisms contributing to sediments. Stable carbon isotopic analysis of whole oils, source rock kerogens and bulk hydrocarbon fractions has been used for several years to aid source-oil and oil-oil correlations.[3] However, since kerogens are usually composed of components derived from different biological sources, bulk carbon measurements by conventional isotope mass spectrometry (following combustion) yields only a composite value. Thus, the complexity of fractionation processes and the information they can convey may be hidden within the bulk isotopic measurements. In order to resolve these separate contributions, the isotopic compositions of individual molecular constituents must be determined. Compound-specific carbon isotope measurements became practical with the recent development of reliable computer controlled gas chromatography-isotope ratio mass spectrometry (GC-IRMS).[4, 5] In this technique, a gas chromatograph is coupled to a combustion furnace and the resultant CO_2 is subsequently continuously analysed by a stable isotope mass spectrometer. The isotopic composition of a specific compound is determined by integrating and ratioing the ion currents of masses 45 ($^{13}CO_2$) and 44 ($^{12}CO_2$) measured during the time that the compound of interest is eluting through the mass spectrometer. Similar isotopic compositions for a series of compounds often implies that they have originated from the same biological sources. Differences in isotopic composition of carbon-bearing substances are usually expressed in terms of the conventional δ-notation giving the permil deviation of the isotope ratio of a sample (sa) relative to that of a standard (st), i.e.

$$\delta^{13}C_{sa} = \left[\frac{(^{13}C/^{12}C)_{sa}}{(^{13}C/^{12}C)_{st}} - 1 \right] \times 10^3 \quad (‰, \text{PDB}) \qquad 4.1$$

The standard commonly used is Peedee belemnite (PDB), a Cretaceous marine carbonate sample, whose $\delta^{13}C$-value defines 0‰ on the δ-scale.

Biological (autotrophic) carbon fixation proceeds by a limited number of assimilatory pathways that transfer carbon dioxide (CO_2), bicarbonate ions (HCO_3^-) and carbon monoxide (CO) from the inorganic carbon reservoir to the biosphere. All pathways of autotrophic carbon fixation entail isotope fractionations of varying magnitude, which, in sum, discriminate against ^{13}C and thus lead to preferential incorporation of isotopically light carbon into cell material. C_3 plants which rely exclusively on the C_3 pathway and constitute the bulk of higher plants, range from about -23 to -34‰ with a mean close to -27‰. Higher plants and some bacteria

utilising the C_4 dicarboxylic pathway are isotopically heavier, displayng average $\delta^{13}C$-values between -12 and -14‰ and a total spread from -6 to -23‰. Compounds derived from methanotrophic bacteria exhibit an unusually light isotopic signature, typically lighter than -50‰. The isotopic signature of eukaryotic algal-derived hydrocarbons are often extremely variable from sample to sample (-8 to -35‰) despite the fact that the C_3 path operates in most algal species and are sensitive to the conditions prevailing in the aquatic environment, such as pH.[6]

In ancient sedimentary systems, the structure and stable isotope ($\delta^{13}C$) content of lipid components may still be (partially) preserved and are often diagnostic of their biological source. GC-IRMS, used in conjunction with the sequential degradation scheme, offers an attractive route to probe compositional fractionation effects that might occur in sedimentary fossil organic matter, due to differences in the mode and extent of incorporation of the different initial biological inputs. As an example, the interesting preliminary results from the sequential degradation of an immature type I oil shale (Göynük) are presented and the implications of these findings, in terms of our present understanding of kerogen formation, are discussed. Göynük oil shale (Oligocene, lacustrine, NW Turkey) represents an important world deposit, with reserves estimated at 10^9 tons, occurring in 100-150 m thick seams underlain by lignites.

EXPERIMENTAL

The bulk geochemical data for the Göynük oil shale[2] and the experimental procedure for the sequential extraction/degradation scheme[1] are reported elsewhere. The Göynük sample used in this study was highly aliphatic (liptinite rich, principally alginite) and was especially organic-rich (ash content < 25%). The aliphatic fractions produced from sequential degradation were analysed by GC-FID using a Varian 3400 gas chromatograph. Separation was achieved with a WCOT 25m fused silica capillary column (0.39mm i.d.) coated with CP-Sil 5CB (0.12µm thickness). Helium was employed as the carrier gas and a temperature program of 50°C (4 mins) to 320°C (12 mins) at 4°C min^{-1} was used. The injection and detection temperatures were both set at 320°C. Compound specific $\delta^{13}C$ measurements were carried out on aliphatic fractions using a VG Isochrom II GC-IRMS instrument. Chromatographic conditions were similar to that used for GC-FID. Urea adduction was employed to separate the straight chain aliphatic components from co-eluting branched/cyclic alkane components, prior to analysis. In general , the reproducibilitiy of multiple analyses for n-alkanes and n-alkenes was excellent (± 0.5‰), after urea adduction had been performed. $\delta^{13}C$ of bulk kerogens (carbonate-free) were also determined by conventional isotope mass spectrometry to provide a suitable reference. All delta values are reported relative to the PDB standard.

RESULTS AND DISCUSSION

DCM extraction of Göynük oil shale released 4.1% w/w of total extract (dry, ash-free basis) of which only a small amount (0.1% w/w) consisted of aliphatic hydrocarbons. The GC-FID trace of the total aliphatic fraction is shown in Figure 1 and it can be seen that the major constituents present were C_{21}, C_{23}, C_{25}, C_{27}, C_{29} and C_{31} n-alkanes (max. C_{23}). An odd carbon predominance of n-alkanes is usually associated with a continental higher plant input; although this is generally in the range n-C_{25} to n-C_{31}. The distribution observed in the case of Göynük may be explained by bacterial reworking of higher plant material during early diagenesis. This can result in the reduction of the chain length of n-alkane components while preserving a noticeable odd over even carbon number predominance. One cannot, however, rule out an input from aquatic organisms. This highlights the speculative nature of interpreting the compound distribution in geological samples. Hopanes were also visible in the GC trace. Monitoring of the m/z 191 fragmentogram by gas chromatography-mass spectrometry (GC-MS) showed that these were hopanes (C_{27} and C_{29}-C_{32}) whose biological configurations had been preserved [17β(H)- for C_{27}; 17β(H), 21β(H)- for C_{29}-C_{32}] indicating a contribution from bacteria. The preservation of the original stercochemistries reflects the immaturity of the sample.

The results of the GC-IRMS analysis for the solvent-extractable n-alkanes isolated from Göynük, as displayed in Figure 3, provided definitive evidence the that n-C_{21}-n-C_{31} alkanes in the bitumen phase of Göynük were not derived from the same parent organisms. The C_{21} and C_{23} n-alkanes had the heaviest isotopic signatures (ca -20‰) and appeared to be derived solely from freshwater algae. The C_{30} and C_{31} members were relatively depleted in ^{13}C (-26.5 and -28.1‰, respectively) indicating an origin from allochthonous higher plant matter. This is consistent with the negative (-7 to -9‰) isotopic fractionation which prevails in the assimilation of atmospheric or dissolved CO_2 in comparison with the assimilation of dissolved HCO_3^- inorganic species by aquatic organisms. The intermediate n-alkanes had isotopic ratios from -22 to -24‰ indicating these chain lengths could be derived from both sources. Thus, analysis of the solvent-extractable alkanes implied an input from diverse sources, including algae, higher plants and bacteria.

Sequential catalytic hydropyrolysis of Göynük (following exhaustive solvent extraction with DCM then pyridine, followed by low temperature catalytic hydrogenation) produced a high yield of aliphatic products from the residual kerogen (approx. 23% w/w on a dry, ash-free basis) and a high overall conversion (over 90% w/w). A bimodal distribution of n-alkanes/n-alk-1-enes (maximum C_{20} and submaximum C_{28}) was produced, with the n-alk-1-enes

paralleling the distribution of their saturated analogues. The GC profile of aliphatics produced from sequential hydropyrolysis are shown in Figure 2 and in each case the n-alk-1-enes elute immediately before the n-alkanes. Interestingly, GC-IRMS indicated that the $\delta^{13}C$ values for the n-alkanes and n-alk-1-enes products were identical within the limits of experimental reproducibility (± 0.5‰) and therefore, were almost certainly derived from the same biological source. These findings are in contrast with the generally accepted rationale that alkyl moieties in the molecular weight range (n-C_{12}-n-C_{20}) are derived principally from phytoplankton while longer-chain n-alkanes (principally n-C_{25}-n-C_{33}) are products of the defuctionalisation of the cuticular waxes of continental higher plants. Thus, a bimodal distribution of aliphatics found in a crude oil or produced from pyrolysis of a sediment may either indicate an input from a single class of organism or may be the result of an input from two or more sources. The observation of isotopic uniformity in the major aliphatic pyrolysis products from a number of kerogens was recently reported[7] and appears to support an origin of the long alkyl chains in these kerogens from aliphatic macromolecular networks derived from the selective preservation of highly resistant aliphatic biopolymers. Non-hydrolysable highly aliphatic biomolecules have been identified in leaf cuticles, sporopollenins and algal cell walls may be a possible source of long-chain n-alkanes (>C_{20}) in sediments and high wax crude oils[8], as well as of shorter chain homologues. The heavy values of $\delta^{13}C$ (ca. -19‰) obtained for n-alkanes produced by sequential hydropyrolysis were typical of those found in some eukaryotic algal species.[9]

It is therefore proposed that Göynük oil shale was largely derived from the highly aliphatic resistant biomacromolecules occurring in the outer cell walls of freshwater green algae, termed *algaenans*. The results for Göynük support the view that kerogens may be composed almost exclusively from selectively preserved or partly altered resistant biomacromolecules rather from random polymerisation and condensation of the remnants from microbial degradation of the initial biomass. This mechanism of kerogen formation is often termed the *Selective Preservation* pathway.

Another point which should be noted from the data displayed in Figure 3 is the fact that the aliphatics produced from sequential hydropyrolysis (ca. -19‰) were distinctly isotopically heavier than the bulk organic carbon of the oil shale (-23.8‰). Even allowing for the presence of vitrinite (around 30% vol), an isotopic balance indicates that liptinite other than lamalginite, with a lighter isotopic signature than the bulk oil shale, must be present. Small quantities of higher plant liptinite can be identified from petrographic analysis of Göynük. GC-IRMS analysis of the n-alkanes (approx. 1.5% w/w on a dry, ash-free basis) released by mild catalytic hydrogenation (320°C, 7.0 MPa H_2) prior to hydropyrolysis, indicated that these were largely derived from higher plant sources (-26 to -28‰ for n-alkanes >C_{22}). Whether this reflects the isotopic composition of the whole hydrogenation product requires further investigation. However, it was observed that with increasing conversion (at higher temperatures) and consequently with higher yields of aliphatic products, the isotopic composition of n-alkanes released by hydrogenation and fixed-bed pyrolysis assumed a less negative (heavier) isotopic composition (approaching the algal isotopic signature). Thus, it was proposed that the more thermally labile n-alkyl components of the kerogen structure were derived largely from allochthonous higher plant material (isotopically light) while the additional n-alkanes released by higher conversion regimes originated from an autochthonous (algal) source (isotopically heavy). As a check, non-sequential hydropyrolysis was conducted on a pyridine-extracted GOS sample. The isotopic signatures of the n-alkanes produced (ca. -24.0‰) were similar to the $\delta^{13}C$ value determined for the bulk carbon of the Göynük kerogen (-23.8‰). Thus, the compositional fractionation effect observed was real and not produced by a kinetic isotope effect associated with hydropyrolysis. The heavy isotopic values of algal-derived aliphatics was likely to reflect low concentrations of dissolved CO_2 in the environment of carbon fixation for Göynük. In such circumstances, many carbon-fixing organisms utilise special pathways (such as assimilation of HCO_3^-) for accumulation of inorganic carbon. The isotopic fractionation effects associated with such pathways are small in comparison to those produced by the regular C_3 pathway, and the resultant organic carbon is relatively enriched in ^{13}C. A thermally-resistant algal backbone for Göynük is consistent with petrographic analysis, which showed alginite (and in particular, lamalginite) to be the dominant maceral.

Additionally, the sequential degradation procedure was applied to the Dunnet torbanite (Type I, Scotland) and a Kimmeridge clay from Dorset (Type II, SW England). Both samples were immature source rocks (< 0.4% R_o) and thus offered a good comparison with Göynük Oil Shale. A lack of any noticeable compositional fractionation for either sample was reflected in the GC-IRMS data for the aliphatic fractions produced (Figures 4 and 5). It was observed that the $\delta^{13}C$ values of aliphatic products from low temperature DCM extraction, mild catalytic hydrogenation and hydropyrolysis were very similar in magnitude, although in the case of Dunnet, the bitumen phase (DCM-extractable) aliphatics were slightly isotopically lighter. A relative lightness of solvent-extractable lipids (usually ca. 2-4‰) relative to the $\delta^{13}C$ value for the bulk kerogen has been widely observed however.[5] The only report of compositional fraction that has appeared in the geochemical literature to date, that parallels that of Göynük, is that of the Messel oil shale (type I, Western Germany). Robinson *et al.*[10] concluded from a study of the extractable lipids that the major sources of organic matter in the Messel oil shale were dinoflagellates and bacteria, especially cyanobacteria. Compound-specific carbon ($\delta^{13}C$) isotope measurements showed that the aliphatic components of the bitumen phase were derived

from diverse sources, including methanotrophic bacteria.[5] However, the analytical pyrolysis products from this oil shale showed a striking similarity to those of algaenans isolated from cultured *Tetraedron Minimum* algae.[11] Scanning electron microscopy (SEM) confirmed that the bulk of the organic matter of Messel oil shale was composed of a *Tetraedron*-type fossilised algal species. While these results for Messel at first seem at variance, it should be recognised that the extractable organic matter of the Messel shale, like that of Göynük, represented at best a few percent of the total organic matter, whilst the kerogen represented 95% or more. In such cases, conclusions based on analyses of solvent extracts probably suffer from a severe bias. Thus, the interpretation of biomarker data with respect to major sources of organic matter should be performed with great caution as it may lead to false interpretations being made.

In the case of Dunnet shale, the similarity in the distribution of aliphatics released at each stage in the degradation scheme (not shown here) together with the observance of isotopic uniformity in the n-alkane/n-alkene products from sequential hydropyrolysis, supported an origin of the aliphatic constituents of this torbanite as being derived almost exclusively from fresh- to brackish-water alga, *Botryococcus*.. A mechanism of selective preservation of resistant aliphatic biopolymers in algal cell walls, as with Göynük, can explain the formation of torbanite. Largeau and co-workers demonstrated the presence of highly resistant biopolymers, termed PRB (Polymère Resistant de *Botryococcus*), in cell walls of Botryococcus.[12,13] Structural and morphological similarities between PRB A and immature Torbanite demonstrated that the resistant polymer was selectively preserved and thus afforded a major contribution to Torbanite formation.[14] Unlike the algal precursor of ultralaminae-type alginite, *Botryococcus* appears to impart a molecular fingerprint (the characteristic n-alkane distribution observed for DS) to the bitumen phase at low maturities. This might be explained by the fact that Botryococcus is anomolously hydrocarbon-rich and can contain up to 76 percent of its dry cell weight as extractable lipids .[15]

CONCLUSIONS

The application of a sequential degradation scheme to an immature oil shale (Göynük), in conjunction with compound specific carbon ($\delta^{13}C$) measurements, highlighted the fact that significant compositional fractionation effects may exist between aliphatic constituents of the free molecular (bitumen) phase and those covalently bound to the insoluble kerogen network. It thus appears, particularly for immature type I source rocks, that the bitumen phase is not necessarily a low molecular weight indicator of the constituents of the bulk kerogen. The isotopic uniformity of the aliphatics released by the high-conversion hydropyrolysis step lends further support to the theory that selective preservation is an important mechanism in kerogen formation.

REFERENCES

1. Love, G.D., Snape, C.E. and Carr, A.D. *Prep. Am. Chem. Soc. Div. Fuel Chem.* **1993**, 38(4), 1281-12 and *Energy & Fuels*, submitted.
2. Love, G.D., Snape, C.E., Carr, A.D. and Houghton, R.C. *Org. Geochem.*, submitted.
3. Schoell, M. In *Advances in Petroleum Geochemistry, Volume 1.* (Eds. J. Brooks and D.H. Welte) pp. 215-245. Academic Press, London, 1984
4. Bjorøy, M., Hall, K. and Jumeau, J. *Trends in Analytical Chemistry* **1990**, 9(10), 331-336.
5. Freeman, K.H., Hayes, J.M., Trendel, J.-M. and Albrecht, P. *Nature* **1990**, 343, 254-256.
6. Schidlowski, M. In *Biological Markers in the Sedimentary Record* (Ed. R.B. Johns) pp. 347-361. Elsevier, Amsterdam, 1986.
7. Eglinton, T.I. *Org. Geochem.* **1994**, 21, 721-736.
8. Tegelaar, E.W., Matthezing, R.M., Jansen, J.B.H., Horsfield, B. and de Leeuw, J.W. *Nature* **1989**, 342, 529-531.
9. Hayes, J.M., Takigiku, R., Ocampo, R., Callot, H.J. and Albrecht, P. *Nature* **1987**, 329, 48-51.
10. Robinson, N., Eglinton, G. and Cranwell P.A. *Chem. Geol.* **1989**, 76,153-173.
11. Goth, K., de Leeuw, J.W., Püttman, W. and Tegelaar, E.W. *Nature* **1988**, 336, 759-761.
12. Largeau, C., Derenne, S., Casadevall, E., Kadouri, A. and Sellier, N. In *Advances in Organic Geochemistry 1985* (Eds. D. Leythaeuser and J. Rullkotter) pp. 1023-1032. Pergamon Press, Oxford, 1986.
13. Berkaloff, C., Casadevall, E., Largeau, C., Metzger, P., Peracca, S. and Virlet, J. *Phytochemistry* **1983**, 22, 389-397.
14. Rohmer, M., Dastillung, M. and Ourisson, G. *Naturwissenschaften* **1980**, 67, 456-458.
15. Maxwell, J.R., Douglas, A.G., Eglinton, G. and McCormick, A. *Phyochemistry* **1968**, 7, 2157-2171.

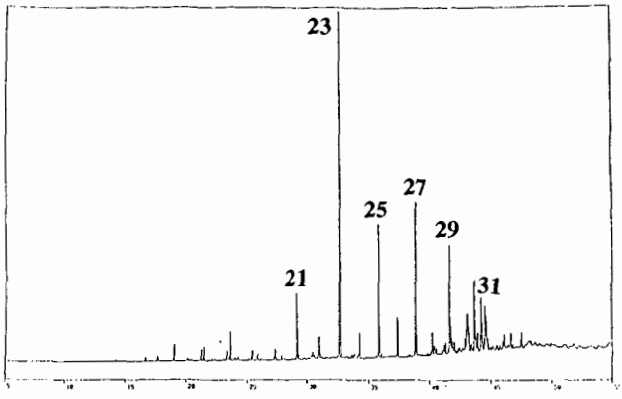

Figure 1. GC-FID trace of the free aliphatic hydrocarbons released by dichloromethane extraction of Göynük Oil Shale.

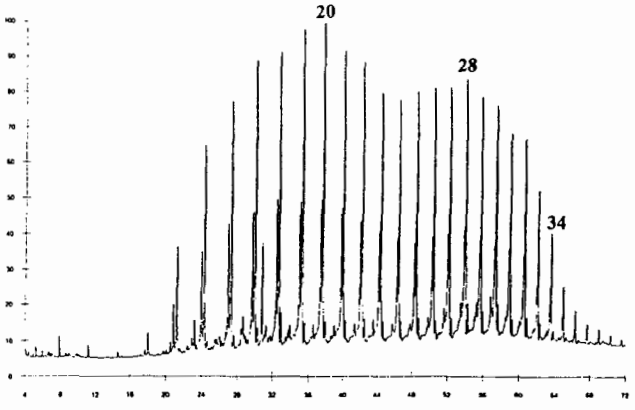

Figure 2. GC-FID trace of the aliphatic hydrocarbons produced from sequential hydropyrolysis of Göynük Oil Shale.

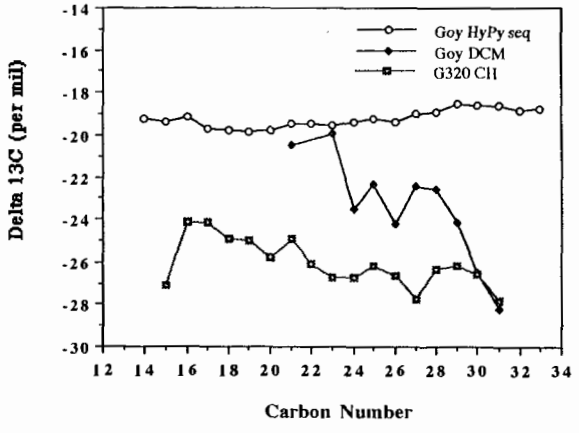

Figure 3. GC-IRMS analysis of *n*-alkanes produced from sequential degradation of Göynük Oil Shale.

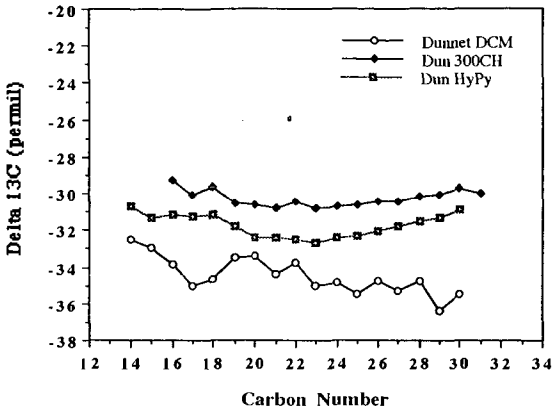

Figure 4. GC-IRMS analysis of n-alkanes produced from degradation of Dunnet torbanite. ($\delta^{13}C$ of bulk TOC = -29.2‰)

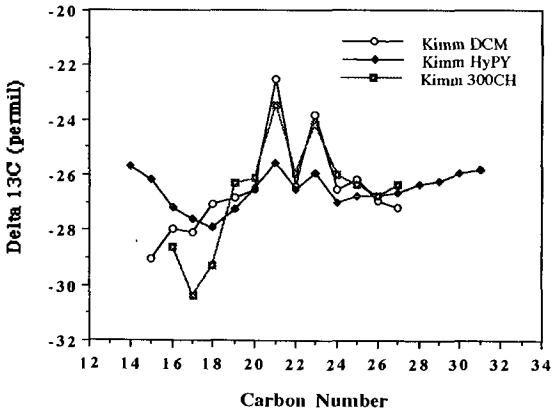

Figure 5. GC-IRMS analysis of n-alkanes produced from degradation of Kimmeridge clay. ($\delta^{13}C$ of bulk TOC = -23.8‰)